Heterogeneous Expression of Immunotherapy Candidate Proteins gp100, MART-1, and Tyrosinase in Human Melanoma Cell Lines and in Human Melanocytic Lesions[1]

Teunis J. de Vries,[2] Aïcha Fourkour, Theo Wobbes, Gerrit Verkroost, Dirk J. Ruiter, and Goos N. P. van Muijen

Departments of Pathology [T. J. d V., A. F., G V., D J. R., G. N. P. v. M.] and Surgery [T. W.], University Hospital, P. O. Box 9101, 6500 HB Nijmegen, the Netherlands.

ABSTRACT

In recent years, it has become evident that T cells can recognize peptides of melanocytic lineage antigens such as gp100, MART-1, and tyrosinase at the tumor cell surface and can subsequently destroy these cells. It is thus feasible to develop immunotherapeutic approaches based on the melanocytic marker profiles of melanoma cells. One of the predictors of the success rate of such a treatment is the extent of positive (target) tumor cells within the lesions of the patient. First, we investigated the presence of these three proteins in 18 human melanoma cell lines using RT-PCR and immunohistochemistry. In 11 cell lines, mRNA and protein of all three markers could be detected; in one cell line, only two markers were present, and six melanoma cell lines showed no evidence for these markers. Secondly, we stained frozen sections of 105 human melanocytic lesions, 13 common nevocellular nevi, 13 atypical nevi, 13 early primary melanomas (Breslow <1.5 mm), 25 advanced primary melanomas (aPM; Breslow ≥1.5 mm), and 41 melanoma metastases (MM) with antibodies against glycoprotein 100, melanoma antigen recognized by T cells, and tyrosinase. In addition, we used the 3,4-dihydroxy-L-phenylalanine reaction to detect tyrosinase enzyme activity as a confirmation of the tyrosinase immunohistochemical results in a subset of the lesions. In the benign lesions, glycoprotein 100 was more prominently expressed in epidermal melanocytes, whereas melanoma antigen recognized by T cells was encountered in all or nearly all dermal melanocytes in all nevocellular nevi and atypical nevus lesions. Tyrosinase was found in a lower percentage of melanocytes, both in the epidermis and in the dermis within these lesions. With regard to heterogeneity of staining within the malignant lesions, we found that 54% (early primary melanomas), 48% (aPMs), and 56% (MM) of the lesions stained within the same staining category for all three proteins studied. Approximately 17% of the aPM and MM lesions did not show positive tumor cells for any of the three proteins. We conclude that a subgroup of patients with high expression should be selected for immunotherapeutic treatment approaches based on the presence of these proteins.

INTRODUCTION

The recent discovery that tumor antigens can evoke tumor-specific immune responses in cancer patients has given new impetus for the possibility of treating patients with immunotherapy (1). Two types of tumor antigens can be distinguished: tumor-specific shared antigens and differentiation- or lineage-specific antigens (2, 3). The first group comprises antigens from the MAGE, BAGE, and GAGE families (4). The second group consists, in the case of melanoma, of proteins such as gp100,[3] MART-1, and tyrosinase (2, 3). Very recently, peptides of gp100 (5, 6), MART-1 (7), and tyrosinase (8–10) have been identi-

fied, which can induce a cellular immune response by presentation via the major histocompatibility complex. Furthermore, T lymphocytes reactive to these proteins could be found in blood of patients with tumor regression (6, 10).

In the past decade, the genes of these three melanocytic lineage-specific proteins have been identified (11–13). Monoclonal antibodies against gp100 have successfully been used in diagnostic pathology (14, 15) and are now implemented in standard melanoma diagnostic pathology practice. In an immunohistochemical study, antibodies against gp100 recognized melanocytes and melanoma cells in the large majority (126 of 134) of the melanocytic lesions (16).

Two proteins also implicated in pigment synthesis and melanosome formation are tyrosinase (13) and the recently cloned MART-1/MelanA (12). Recently, antibodies against these proteins, which can be used in immunohistochemical stainings of melanocytic lesions, have been described (17–19). With regard to coexpression of either MART-1 or tyrosinase with gp100, only a small number of melanocytic lesions were studied.

One of the main predictors of successful immunotherapeutic treatment is the extent of expression of the immunization proteins in the tumor lesions, because absence or low expression of these proteins can lead to a poor immune response (21). To get more insight into the relevance of immunotherapeutic approaches against gp100, MART-1, and tyrosinase in melanoma patients, it is of great importance to know more about the abundance of expression of these proteins in a large group of melanocytic lesions. In the present study, we therefore documented the presence of gp100, MART-1, and tyrosinase in 18 melanoma cell lines and in 105 melanocytic lesions covering all stages of melanocytic tumor development.

MATERIALS AND METHODS

Cell Lines. Eighteen human melanoma cell lines were used in this study: BLM, 1F6, 1F6-m, 530, Mel57, MV1, MV3, A375P, A375M, Omel2, Bowes, MD3A, M24met, E10, 518A2, 603, MZ2-MEL-3.0, and SK-mel-28. Besides these cell lines, BLM cells (negative for all three melanocytic markers studied) transfected with either gp100, MART-1, or tyrosinase cDNA were used as positive controls for RT-PCR and immunocytochemistry. Three nonmelanoma cell lines were used as negative controls: UMSCC2 (head and neck squamous cell carcinoma), A431 (cervical carcinoma), and CaCo2 (colon carcinoma). BLM, 1F6, 1F6-m, 530, Mel57, MV1, and MV3 were from the Department of Pathology, University Hospital (Nijmegen, the Netherlands), and have been described extensively (22, 23). Gp100-, MART-1-, and tyrosinase-transfected BLM cells were from Alex Bakker, Department of Tumorimmunology, University Hospital (Nijmegen, the Netherlands). Melanoma cell lines MD3A, E10, 518A2, and 603 were established at the Department of Clinical Oncology, University Hospital (Leiden, the Netherlands). Cell lines Omel2 (Dr. H. Spits, Division of Immunology, Netherlands Cancer Institute, Amsterdam, the Netherlands), M24met (Dr. B. Mueller, The Scripps Institute, La Jolla, CA), MZ2-MEL-3.0 (Dr. F. Brasseur, Ludwig Institute, Brussels, Belgium), and UMSCC2 (Dr. T. Carrey, University of Michigan, MI) were provided kindly from the institutions where the cell lines were characterized. Cell lines A375, Bowes, SK-mel-28, A431, and CaCo2 were obtained from the American Type Culture Collection (Rockville, MD).

Cell lines were cultured and trypsinized as described previously (20). One

751bp► gp100

384bp► MART-1

254bp► tyrosinase

β₂m

136bp►

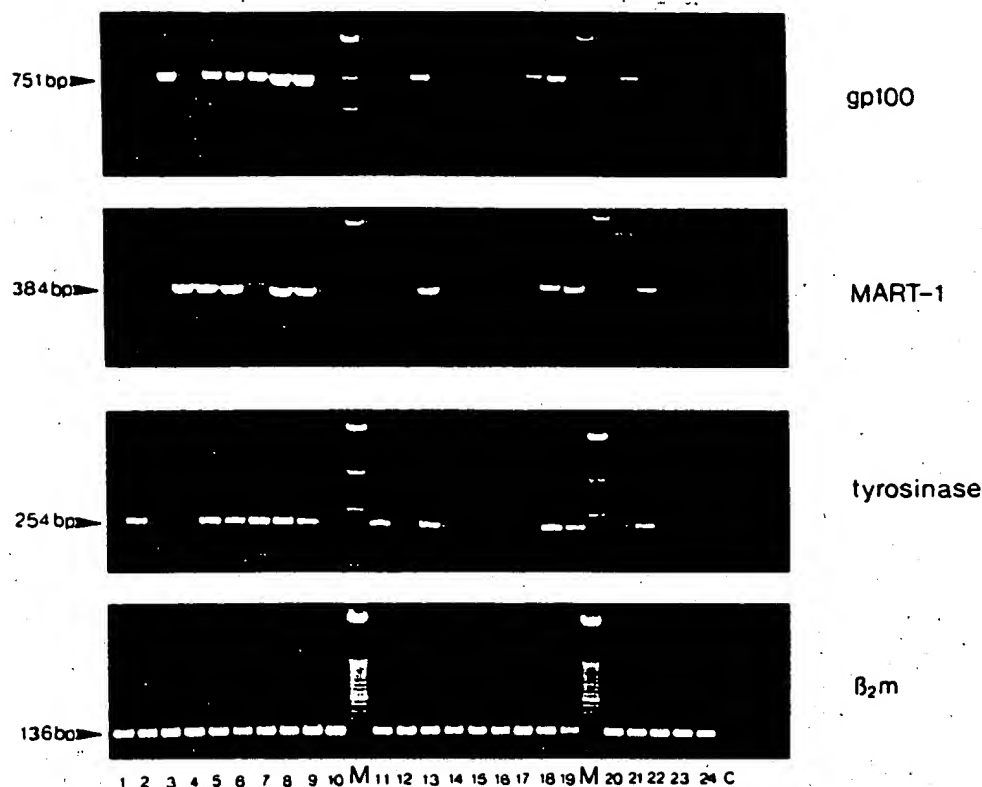1 2 3 4 5 6 7 8 9 10 M 11 12 13 14 15 16 17 18 19 M 20 21 22 23 24 C

Fig. 1. Expression of gp100, MART-1, or tyrosinase mRNA in melanoma and nonmelanoma cell lines. Cell lines were subjected to RNA isolation and RT-PCR with primers specific for gp100, MART-1, tyrosinase, and β₂-microglobulin (control). PCR products were analyzed subsequently on an ethidium bromide-stained agarose gel. *Lane 1*, BLM. *Lane 2*, BLM-tyrosinase. *Lane 3*, BLM-gp100. *Lane 4*, BLM-MART-1. *Lane 5*, 1F6. *Lane 6*, 1F6-m. *Lane 7*, 530. *Lane 8*, Mel57. *Lane 9*, MV1. *Lane 10*, MV3. *Lane 11*, A375P. *Lane 12*, A375M. *Lane 13*, Omel2. *Lane 14*, Bowes. *Lane 15*, MD3A. *Lane 16*, M24met. *Lane 17*, E10. *Lane 18*, 518A2. *Lane 19*, 603. *Lane 20*, M72-MEL-3.0. *Lane 21*, SK-mel-28. *Lane 22*, UMSCC2. *Lane 23*, A431. *Lane 24*, CaCo2. C, negative control (water put in the RT reaction, and later in the PCR reaction). M, 50-bp ladder. PCR was performed twice, and the same results were obtained.

part of the cells was used for cytospin preparations, and the other part was used for RNA isolation.

RNA Isolation and RT-PCR. RNA was isolated using a commercial kit (RNeasy Mini Kit; Qiagen, Hilden, Germany) following the manufacturer's instructions. One μg of total RNA was reverse transcribed in a 50 mM Tris-HCl buffer (pH 8.3) containing 75 mM KCl, 3.0 mM MgCl₂, 10 mM DTT, 220 μM of each deoxynucleotide triphosphate, 50 pmol of oligodeoxythymidylate, and 10 units of AMV (Promega, Leiden, the Netherlands). For PCR, each vial contained 75 mM Tris-HCl (pH 9.0), 20 mM (NH₄)₂SO₄, 0.01% Tween (w/v), 2 mM MgCl₂, 50 pmol of each specific primer, 0.2 units of Thermoperfectplus DNA polymerase (Integro, Zaandam, the Netherlands), and 0.1 μg of reverse-transcribed RNA.

The following primers were used: for gp100, 5'-GCTTGGTGTCTCAAG-GCAACT-3' (sense) and 5'-CTCCAGGTAAGTATGAGTGAC-3' (antisense), yielding a 751-bp product; for MART-1, 5'-ATGCCAAGAGAAGAT-GCTCAC-3' (sense) and 5'-AGCATGTCTCAGGTGTCTCG-3' (antisense), yielding a 384-bp product; and for tyrosinase, 5'-TTGGCAGATTGTCTG-TAGCC-3' (sense) and 5'-GCTATCCCAGTAAGTGGACT-3' (antisense), yielding a 254-bp product. The tyrosinase primers are also known as HTYR1 and HTYR4 (24).

Primers for the housekeeping gene β₂-microglobulin were used to control the amplifiability of the mRNA used. The following primers were used: 5'-CTCGCGCTACTCTCTCTTTCT-3' (sense) and 5'-TGTCGGATTGAT-GAAACCCAG-3' (antisense), yielding a 136-bp product.

Each PCR was preceded with a hot start of 5 min at 94°C, and each PCR was terminated with an end extension for 10 min at 72°C. The specific PCR conditions were: for gp100, 1 min at 94°C and 1.5 min at 60°C for 30 cycles; for MART-1, tyrosinase, and β₂-microglobulin, 30 s at 94°C and 1 min at 60°C for 30 cycles.

The PCR products were visualized on a 2% agarose gel containing ethidium bromide.

Melanocytic Lesions. Representative tissue samples were freshly received from cutaneous melanocytic lesions excised from patients at University Hospital (Nijmegen, the Netherlands) and from patients at University Hospital (Würzburg, Germany). They were snap frozen in liquid nitrogen and stored at −80°C until sectioning. Four-μm cryostat sections were cut from 105 lesions comprising all stages of melanocytic tumor progression: 13 NN, 13 AN, 13 ePMs (Breslow index <1.5 mm), 25 aPMs (Breslow index ≥1.5 mm), and 41

distant MM. H&E-stained paraffin sections of the same lesions were used for classification of the lesions.

Antibodies and Immunohistochemistry. NKI-beteb (16) was used as antibody against gp100. C-10 (19) and A103 (18) were used as antibodies against MART-1, and T311 (17) was used as an antibody against tyrosinase. The anti-CD3 antibody WT32 (25) was used as a negative control. Monoclonal antibodies 2A7, 4G2, and 6G6 against HMW-MAA (26) were used in the fluorescence-activated cell sorting experiments. Three consecutive sections of the melanocytic lesions and four cytospins of each cell line were stained in immunohistochemistry. An avidin-biotin complex-peroxidase method was used (20, 27). Antibody binding was visualized with 3-amino-9-ethylcarbazole as a substrate.

Flow Cytometric Analysis. The method has been described in detail previously (20). Briefly, 10⁵ trypsinized cells were incubated at 4°C with monoclonal antibodies against HMW-MAA. An incubation with irrelevant antibody WT32 (25) served as a negative control.

Score. For each section, the percentage of positive melanocytic cells was estimated. Each section was assigned to one of the following percentage categories: 0, 1–5, 5–25, 25–50, 50–75, and 75–100%. Positive melanocytic staining was scored when at least 1% of the melanocytic cells stained. The scoring was performed by three observers (T. J. d. V., G. N. P. v. M. and D. J. R).

DOPA Reaction. Parallel to the immunohistochemical staining, we used the enzyme histochemical DOPA reaction to confirm tyrosinase activity in a subset of the lesions. Four-μm cryostat sections were cut from 10 lesions (2 NN, 2 AN, 3 aPMs, and 3 MM) with known (presence or absence) immunoreactivity for tyrosinase. Parallel sections were stained for immunohistochemistry and for the DOPA reaction. One mg/ml DOPA (Sigma Chemical Co., Bernhem, Belgium) was dissolved in 0.1 M phosphate buffer (pH 7.4). The reaction was stopped after 4 or 6 h. A 0.1 M phosphate buffer, in which the substrate was omitted, served as a negative control.

RESULTS

Expression of gp100, MART-1, and Tyrosinase in Human Cell Lines. We studied the expression of gp100, MART-1, and tyrosinase in a panel of 18 melanoma cell lines and 3 nonmelanoma cell lines

Fig 2. Heterogeneous expression of gp100. MART-1. and tyrosinase in the 530 melanoma cell line. Cytospins of the 530 cell line were stained with antibodies against gp100 (a), MART-1 (antibody A103 was used) (b), or tyrosinase (c). As a negative control, a CD-3 antibody WT32 was used (d). Arrowheads, positive cells. Magnification: bar. 50 μm
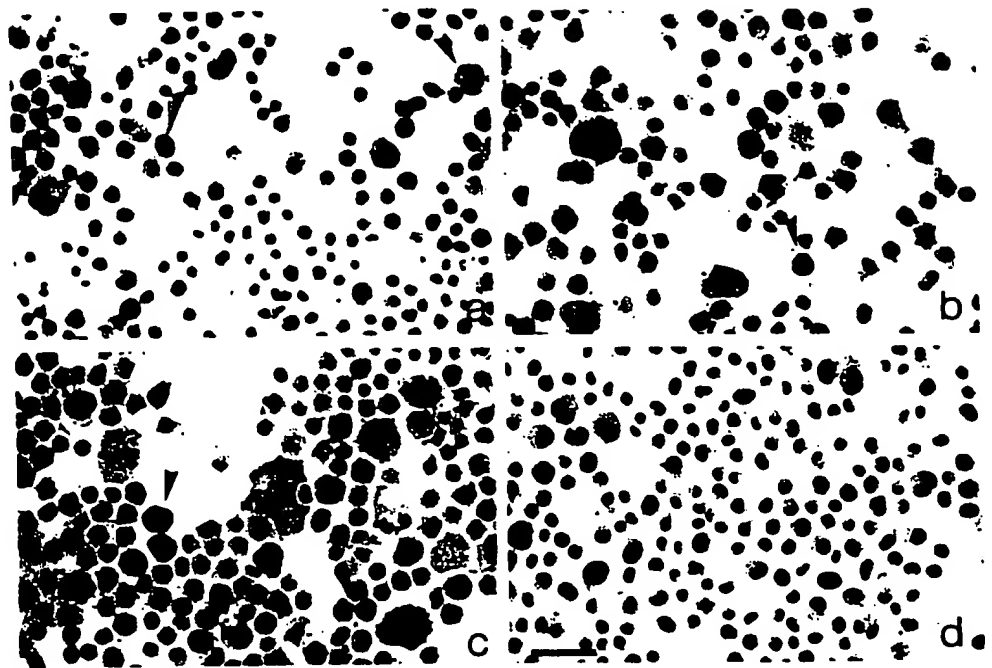
both at the mRNA (Fig. 1) and at the protein level (Fig. 2). After RT-PCR. the gp100-. MART-1-, and tyrosinase-transfected BLM cell lines (positive controls) specifically showed the products of the melanoma markers with which they had been transfected. Also, these three positive control cell lines showed specific immunocytochemical staining with antibodies against these markers (Table 1).

In general, the RT-PCR data perfectly matched the immunohistochemical data, except in the case of cell line A375P. in which a slight PCR band for gp100 and MART-1 could be visualized. whereas no immunoreaction could be observed (Table 1). This could be due to differences in sensitivity between the techniques. mRNA for gp100, MART-1, and tyrosinase was present in 11 of 18 melanoma cell lines.

In 6 of 18 melanoma cell lines. none of these three melanoma marker mRNAs could be detected with RT-PCR. To further confirm the melanocytic status of these six cell lines. the presence of HMW-MAA was investigated with flow cytometry. Three of these cell lines (of six) showed HMW-MAA fluorescence (Table 1). The cell line MZ2-MEL-3.0 was the only cell line in which expression of gp100 and tyrosinase mRNA did not coincide with the presence of MART-1.

In nonmelanoma cell lines and in the melanoma cell lines in which no gp100 protein could be detected. a very slight gp100 band could be visualized after overexposure of the ethidium bromide gel. Although established cell lines were used. heterogeneity of immunocytochemical staining (e.g.. cells with absence and cells with presence of the

Table 1 *Expression of gp100. MART-1. and tyrosinase in human melanoma and nonmelanoma cell lines*[a]

	gp100		MART-1		Tyrosinase		β2m PCR	HMW-MAA FCM
	PCR	ICC	PCR	ICC	PCR	ICC		
Melanoma cell lines								
BLM	−	−	−	−	−	−	−	−
BLM-tyrosinase	−	−	−	−	−	−•	−	ND
BLM-gp100	+	+	−	−	−	−	−	ND
BLM-MART-1	−	−	+	+	−	−	−	ND
1F6	−	−	+	+	+	−	−	−
1F6m	−	−	+	−	+	−	−	−
530	+	−•	+	−•	+	+	−	−
Mel57	+	−	+	−	+	−	−	ND
MV1	+	−	+	−	+	−	−	−
MV3	−	−	+	−	+	−•	−	−
A375P	(−)	−	(−)	−	(−)	−	−	−
A375M	(−)	−	(−)	−	(−)	−	−	−
Omel2	−	−	−	−	−	−	−	−
Bowes	−	−	−	−	−	−	−	−
MD3A	−	−	+	−	+	−	−	−
M24met	−	−	+	−	+	−	−	−
E10	−	−	−	−	−	−	−	−
518A2	(−)	−•	−	−•	−	−•	−	−
603	+	−	+	−	+	−	−	ND
MZ2-MEL-3.0	+	−•	−	−	+	−•	−	ND
SK-mel-28	−	−	+	−	+	−	−	−
Non-melanoma cell lines								ND
UMSCC2	−	−	−	−	−	−	−	−
A431	−	−	−	−	−	−	−	−
CaCo2	−	−	−	−	−	−	−	−

[a] β2m β2-microglobulin. ICC. immunocytochemistry. FCM. flow cytometry −. no immunocytochemical staining or PCR band was observed. −. PCR or homogeneous immunohistochemical positivity was observed. −•. heterogeneous expression was observed in immunocytochemistry. (−). only a slight PCR band could be visualized: ND. not determined
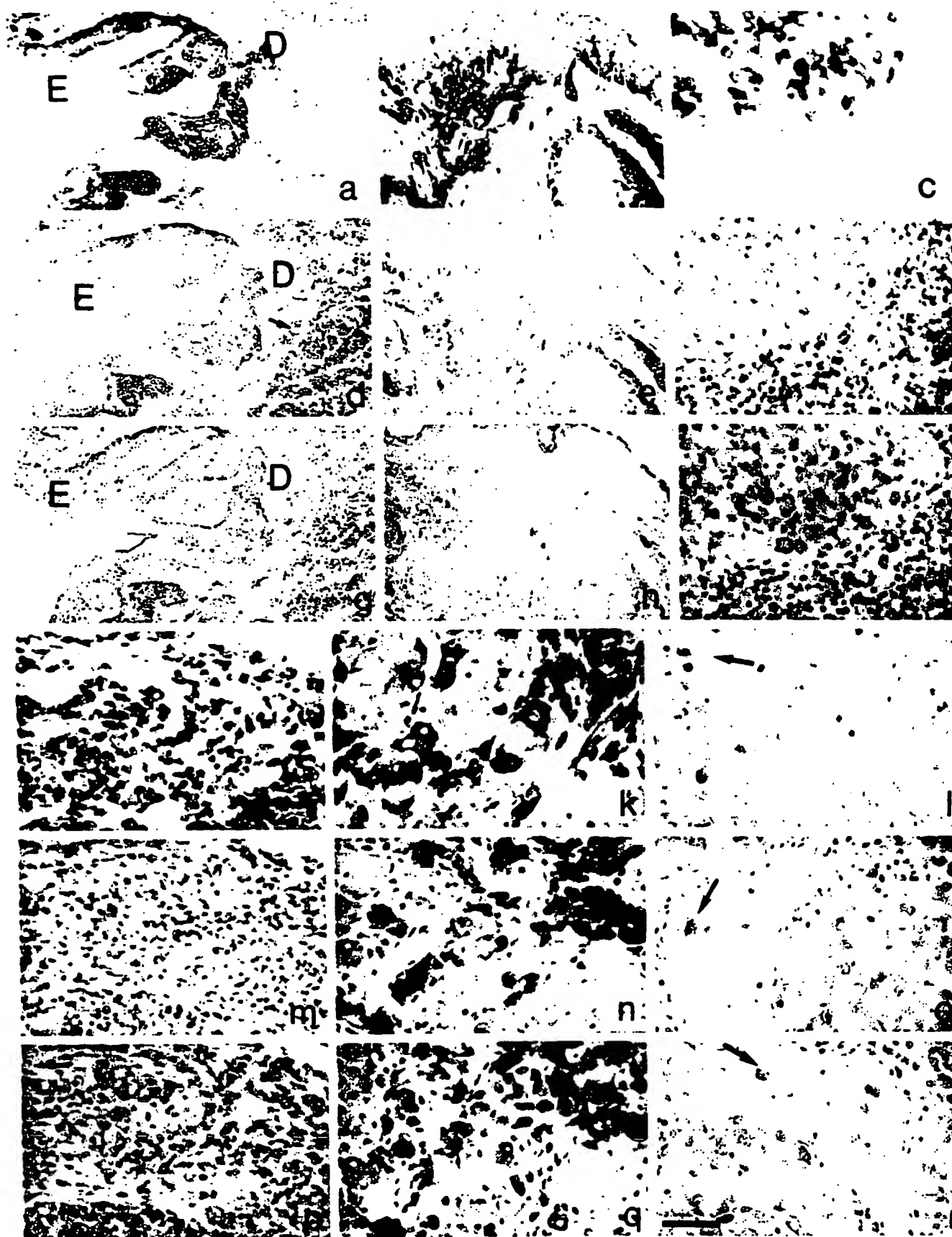
Fig. 3. Immunohistochemical staining for gp100, MART-1, and tyrosinase on melanocytic lesions. Three successive sections of 105 melanocytic lesions were stained with antibodies against these melanocytic lineage markers. Examples of one AN (a, d, and g), one ePM (b, e, and h), and four MM (c, f, and i; j, m, and p; k, n, and q; and l, o, and r) are shown. a–c and j–l, gp100 staining; d–f and m–o, MART-1 staining; g–i and p–r, tyrosinase staining. a, d, and g, staining of an AN: melanocytes in the epidermis (E) are stained, nevomelanocytes in the dermis (D) are negative, except for MART-1 (d, arrow). b, e, and h, staining of a melanotic ePM: note that no tyrosinase immunoreactivity could be detected (h) in this lesion. c, f, and i, equal staining of gp100, MART-1, and tyrosinase in this MM: most tumor cells (75–100%) stained. j–r, three examples of MM with heterogeneous staining. j, m, and p, gp100 (j) and tyrosinase (p) present in all tumor cells and total absence of MART-1 (m). k, n, and q, heterogeneous staining for gp100 (k); there are hardly any MART-1
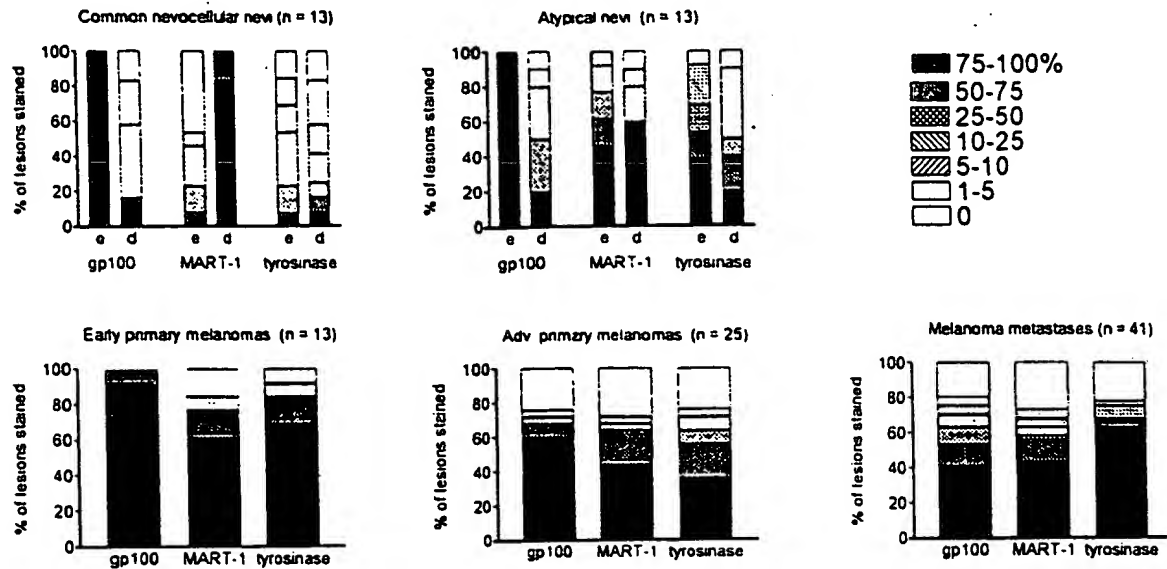
Fig. 4. gp100, MART-1, and tyrosinase expression in 105 melanocytic lesions, expressed as a percentage of immunoreactive cells. Each immunohistochemically stained lesion was assigned to a percentage of positive melanocytic cells. Within the NN and AN, we scored the epidermal (e) and dermal (d) melanocytic cells separately. All benign lesions contained an epidermal part of the lesion: 12 NN and 10 AN revealed an identifiable dermal part as well.

antigen within the same cytospin) was observed in five cell lines (Table 1). An example of such heterogeneity is shown in Fig. 2.

For all three markers, no mRNA or protein could be detected in the three nonmelanoma cell lines included in this study.

Expression of gp100, MART-1, and Tyrosinase in Human Cutaneous Melanocytic Lesions. One hundred five melanocytic lesions were stained with antibodies against gp100, MART-1, and tyrosinase. Immunohistochemical staining of melanocytic cells in benign lesions and tumor cells in malignant lesions were scored. Some representative examples are shown in Fig. 3. The scoring results are depicted in Fig. 4.

All three antigens could be detected in normal melanocytes that are scattered throughout the epidermis. Within the group of benign lesions (NN – AN), we distinguished between staining of epidermal and dermal nevomelanocytes. It was striking that gp100 was primarily found in (nearly) all of the epidermal nevomelanocytes. MART-1 and tyrosinase were encountered in a lower percentage within the epidermal melanocytes. Immunoreactivity against gp100 and tyrosinase was present in a low percentage of dermal nevomelanocytes and diminished with the depth of the lesion. Contrasting this, MART-1 was found in nearly all dermal nevomelanocytes, regardless of the depth of the lesion (Fig. 4). Both MART-1 antibodies showed identical staining. A typical example of the staining aspects for the different antigens in a benign lesion is depicted in Fig. 3, a, d, and g.

Most ePMs contained 75–100% positive tumor cells for all three markers studied, whereas fewer tumor cells stained within the groups of aPMs and MM. An unexpected finding was that in some pigmented tumors, we could not visualize tyrosinase immunoreactivity (Fig. 3, b, e, and h). This was confirmed with the DOPA reaction, in which hardly any tyrosinase enzyme activity could be detected (not shown).

An important question for immunotherapeutic application and immunopathological diagnosis of melanoma is whether these antigens are equally abundant or whether there are great differences in expression of these antigens within the lesions. We calculated the degree of concurrence of staining for the three antigens (equal percentages of

stained tumor cells within one lesion). Within the group of ePMs, 7 of 13 (54%) stained within the same category; within the aPMs, 12 of 25 (48%) stained within the same category; and within the MM, 23 of 41 (56%) stained within the same category. An example of a MM in which most tumor cells were positive (75–100% category) is shown in Fig. 3, c, f, and i. Three striking examples of heterogeneity of staining within the melanoma metastases group are shown in Fig. 3, j–r.

Within the group of aPMs, 4 of 25 (16%) showed absence of the three antigens. For the individual components, these numbers were higher: six (gp100), eight (MART-1), and seven (tyrosinase) lesions were negative. The group of MM displayed a similar pattern: 7 of 41 lesions (17%) were negative for all three antigens. These numbers were 7 (gp100), 10 (MART-1), and 9 (tyrosinase), when looking at one melanocytic lineage marker only.

DOPA. The DOPA reaction was performed on a subset (10) of the lesions to compare tyrosinase immunoreactivity with tyrosinase enzyme activity. This series included cases with extensive, scattered, and no tyrosinase immunoreactivity. Four successive sections (including two negative controls) were used for these incubations, two for immunohistochemistry and two for registration of enzyme activity. Within benign lesions, enzyme activity and immunoreactivity were visualized in individual melanocytes and in nests of melanocytes in the epidermis and in melanocytes of the upper dermis (results not shown). Codistribution of immunoreactivity and enzyme activity was also eminent in the malignant cases. An example is shown in Fig. 5.

DISCUSSION

In the present paper, we made an inventory of the expression of gp100, MART-1, and tyrosinase in 18 human melanoma cell lines and in 105 human melanocytic lesions. We studied melanoma cell lines with RT-PCR with primers specific for the mRNAs and with immunocytochemistry on cytospins of these cell lines. With RT-PCR, we found that transcription of gp100 coincides with transcription of MART-1 and tyrosinase in 12 of 18 cell lines. Immunocytochemistry

Fig. 5 Concurrence of tyrosinase protein by immunohistochemical localization and enzyme activity. An example of an aPM is shown, four successive sections were used. a, immunohistochemical staining with the T311 antibody; b, immunohistochemistry-negative control; c, DOPA reaction for the detection of tyrosinase enzyme activity; d, DOPA reaction-negative control. Magnification bar, 50 μm.
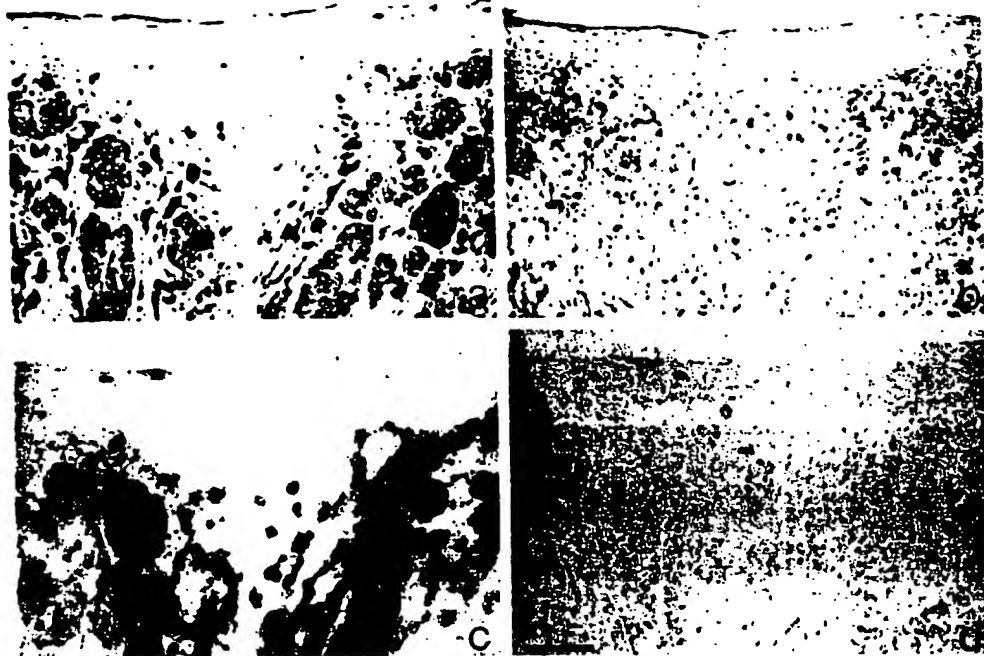
revealed heterogeneity of expression in some of the cell lines. This heterogeneity was described previously by us (22) for some of the melanoma cell lines also included in this study. This heterogeneity may be due to clonal differentiation of the cell lines or to differences in the cell cycle of the cells, resulting in a temporarily shutting down of synthesis of some proteins involved with pigment formation. The gp100 RT-PCR revealed a very slight band on ethidium bromide-stained agarose gels, also in the cell lines negative for gp100 protein. Apparently, a very low level of "illegitimate" transcription of gp100 is present in cell lines, as revealed recently by us (28) and others in human tissue samples and cell lines of nonmelanocytic origin (29). Similar low levels of transcription could be detected for the mouse homologue of gp100 in mouse tissues and cell lines (30). This low level of transcription in nonmelanoma cells implicates that a gp100 RT-PCR cannot be used as an additional candidate, next to the specific and well-established tyrosinase RT-PCR (24, 31), for the detection of low levels of tumor cells in blood or in putatively involved lymph nodes. Half of the melanoma cell lines that were negative for the three markers did express detectable levels of HMW-MAA; thus, in part, the melanocytic origin of these cell lines was confirmed.

The human melanocytic lesions displayed interesting staining aspects with the antibodies used. Within NN and in AN, a strong MART-1 staining was present in a high percentage of dermal nevomelanocytes, regardless of the depth of the melanocytes. The epidermal nevomelanocytes displayed more gp100 immunoreactivity than MART-1 or tyrosinase immunoreactivity. Heterogeneity of staining with the antibodies used was observed in the malignant lesions. Concordance of expression, defined as staining within the same category of percentage of positive cells with all three antibodies used, was observed in approximately 50% of the primary melanomas and metastasis lesions. Thus, heterogeneity (e.g., stainings within different categories) was observed in the remaining 50% of these lesions. In pathology practice, it means that using antibodies recognizing more than one melanocytic lineage protein increases the likelihood of diagnosing melanoma cells in a suspected lesion. An example of this has been worked out in "Results": in the group of aPMs, six cases were gp100 negative, eight were MART-1 negative, and seven were

tyrosinase negative. Only four cases were negative for all three components. Similar results were found by Marincola et al. (19) who found gp100-positive and MART-1-negative patient samples and vice versa and by Chen et al. (17) who found heterogeneity of staining between gp100, tyrosinase, and TRP-1 in 16 melanoma specimens. Using multiple markers increases the sensitivity of detection, as recently also found by Hoon et al. (31) in a RT-PCR assay for the detection of circulating melanoma cells in blood from tumor-bearing patients.

Recently, false-positive results with the widely used gp100 antibody HMB-45 have been reported (32, 33). Therefore, additional markers for the diagnosis of melanoma cells are required as a confirmation or rejection of the results. This article shows that tyrosinase and MART-1 can be used as additional markers. Especially when these antibodies can be used on paraffin-embedded tissues, they can be introduced in general pathology practice.

The observed heterogeneic staining for the three melanocytic lineage markers has great implications for immunotherapeutic devices. It seems wise to implement vaccination strategies based on total melanoma cells or on mixtures of melanoma-associated antigens. In our series, we found examples of 100% staining for two antigens and no staining for the third (e.g., Fig. 3, j, m, and p). Most likely, vaccination of this patient with only MART-1 peptides would have no effect on this metastasis lesion. To date, treatment of metastatic melanoma is hardly successful, and survival times of these patients are very short. It is important to emphasize that a subset of approximately one-sixth of the aPM and MM group does not express any of the investigated melanoma markers. In yet another subset, only a low percentage of tumor cells expresses gp100, MART-1, and tyrosinase. With novel immunotherapeutic approaches, it will be important to carefully map the melanoma antigens by immunohistochemistry on biopsy material from the melanoma patient before treatment is started.

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